Activity 3

Superbugs: Ä An Evolving ConcernÄ

At a Glance

Focus: Students investigate the growth of bacteria in the presence of antibiotics and use the results to explain a case of antibiotic-resistant tuberculosis, pre sented in a CD-ROM-based interview.

Major Concepts: The re-emergence of some diseases can be explained by evo lution of the infectious agent (for example, mutations in bacterial genes that confer resistance to antibiotics used to treat the diseases).

Objectives: After completing this activity, students will

- be able to explain how antibiotic treatment results in populations of bacteria that are largely resistant to the antibiotic and
- describe inappropriate and / or questionable uses of antibiotics.

Prerequisite Knowledge: Students should be familiar with bacterial growth and with evolution by natural selection.

Basic Science-Public Health Connection: In this activity, students learn that the evolution of antibiotic resistance among bacteria observed in laboratory experi ments occurs in the natural environment as well, and that such evolution has serious consequences for the effectiveness of treatments for some diseases.

Introduction

In 1943, penicillin was introduced as the “magic bullet” for curing many infec tious diseases. By 1946, however, approximately 14 percent of *Staphylococcus aureus* strains isolated at a London hospital were resistant to penicillin. Today, scientists estimate that more than 95 percent of all *S. aureus* strains are peni cillin-resistant.

After the introduction of penicillin, additional antibiotics were rapidly isolated and developed, including streptomycin and the tetracylines. Today, there are more than 100 antibiotics available. Nevertheless, some strains of at least three bacterial species (*Enterococcus faecium, Mycobacterium tuberculosis, Pseudomonas aeruginosa*) are resistant to all of these antibiotics, and health care workers fear the time is rapidly approaching when more deadly organisms escape the effects of all known antibiotics.

The primary reason for the increase in antibiotic resistance is evolution. When mutant genes arise that make a bacterium less sensitive to an antibiotic, that bacterium survives and produces descendants in an environment rich in antibi otics. That is, the process of natural selection operates. Multiple mutations may be required to result in fully resistant bacteria. However, once resistant genes appear, bacteria have a variety of mechanisms for exchanging those (and other) genes both within and across species. These mechanisms include conjugation,

transformation, transduction, and transposon-mediated exchange. This exchange allows for "accelerated evolution" of bacterial species (accelerated in the sense that random mutations that result in antibiotic resistance need not occur in every individual bacterium, nor even in every species of pathogen, but can simply be acquired from another organism).

This activity invites students to explore one reason for the re-emergence of some infectious diseases: the evolution of antibiotic resistance among pathogens. In Activity 4, *Protecting the Herd*, students explore another reason for the re-emergence of infectious diseases.

Materials and Preparation

You will need to prepare the following materials before conducting this activity:

- Master 3.1, *Bacterial Growth Experiment* (make 1 copy per student)Ä
- Master 3.2, *Discussion Questions for the Bacterial Growth Experiment* (make 1 copyÄ per student)
- Master 3.3, *Debi's Story: Explaining What Happened* (make 1 copy per student)
- Master 3.4, *Antibiotic Concerns* (make 1 per team)
- *Emerging and Re-emerging Infectious Diseases* CD-ROM (1 per team)

Students complete this activity across a five-to-seven day period. You will need to prepare the materials for the laboratory exercise. Ordering information and preparation directions are on page 64, immediately following the activity.

Information about the safe use of microorganisms in classrooms, including lists of organisms considered safe for students at various levels of school, can be found at: http://www.science-projects.com/safemicrobes.htm. A number of leaders in infectious diseases, including scientists from NIH, contributed to the Web site. *Pseudomonas fluorescens*, the organism used in the laboratory exercise in this activity, is included on the list of microorganisms considered appropriate for students in grades 9 or higher. Nevertheless, experts acknowledge that people who are immunocompromised may be at risk for infection by organisms that do not affect healthy individuals. We recommend that you read a statement such as the following to your classes before beginning the activity:

> *Pseudomonas fluorescens*, the bacteria used in the laboratory exercise you will begin soon, does not cause disease in healthy people. However, people who have weakened immune systems should not have contact with most microorganisms or with people who handle those organisms. Your immune system may be weakened if you are undergoing antibiotic therapy, if you are taking immunosuppressive drugs or drugs for cancer treatment, or if you have AIDS or are HIV-positive. If you have a weak ened immune system for these or any other reasons, let me know and I will provide you with an alternative experience that is safer for you.

Students who should not participate in the laboratory exercise can view a video demonstration of it on the CD-ROM as described in the following paragraphs.

They can rejoin the class in Day 3 of the activity, after the other students have recorded their results and discarded their bacterial cultures.

If you do not have the time or facilities for conducting the laboratory exercise, you will need only one day to complete this activity. Complete Steps 1 to 3, Day 1, and then have students view a video demonstration of the laboratory exercise, *Bacterial Growth Experiment* on the *Emerging and Re-emerging Infectious Diseases* CD-ROM. Students will need copies of Master 3.1 to help them follow the steps in the demonstration. Then move to Day 3 of the activity.

Follow the instructions on page 31 to load the CD-ROMs into the computers students will use.

Note to teachers: If you do not have enough computers equipped with CD-ROM drives to conduct this activity, you can use the print-based alternative. To view and print the instructions and masters for this alternate activity, load the CD onto a computer and click the Print button on the main menu screen. The computer will display a screen showing the resources available for printing from the CD; click on the button labeled Non-CD Lesson Plan from the choices available for Activity 3, *Superbugs: An Evolving Concern*. This will reveal the lesson plan and the masters for the alternate, non-CD-based lesson. Click Print again to print these resources.

Procedure

DAY 1 (5 to 7 days before Day 3 of the activity)

1. **Remind students of the theory of evolution by natural selection and tell them that a powerful feature of theories is that they lead to hypotheses that can be experimentally tested.**

   Students should be able to state the basic elements of the theory of evolution: (1) there is variation among the individuals in a population; (2) some of these differences can be inherited; (3) some individuals will be better adapted to their environment than others; (4) the better adapted individuals will reproduce more successfully; and (5) thus, the heritable characteristics that make individuals better adapted will increase in frequency in the population.

2. **Organize students into teams of three and challenge the teams to use their understanding of evolution by natural selection to write a hypothesis about what will happen in a population of bacteria after growing for several generations in the presence of an antibiotic.**

   If students have difficulty with this, stimulate their thinking by asking questions such as, “What effect does an antibiotic usually have on a bacteria? Do you know of cases in which that effect did not occur? What does that suggest about variations that exist in the bacteria population? Which bacteria survived? What trait did they pass on to other progeny?”

3. **Convene a class discussion in which you ask several teams to share the hypotheses they developed. Challenge the class to work together to refine them into one hypothesis similar to the following:**

If a bacterial culture is grown in a medium containing an antibiotic, then after several generations, all of the bacteria in the culture will be resistant to the antibiotic.

4. **Tell students that they will conduct an experiment to test this hypothesis and explain that they will also consider the implications of their results for controlling infectious diseases in an activity the following week. Then distribute Master 3.1, *Bacterial Growth Experiment*, and instruct students to complete Steps 1 through 4 with their team members.**

   Emphasize that for safety reasons as well as the success of their experiments, students must use aseptic techniques. If students are not familiar with aseptic techniques for handling bacterial cultures, you will need to demonstrate them. Alternatively, you can have your students view the "Day 1" video segment of *Bacterial Growth Experiment*, which shows students using aseptic techniques as they prepare the initial cultures in the experiment.

DAY 2 (2 to 3 days before Day 3 of the activity)

1. **Direct teams to complete the remaining steps on *Bacterial Growth Experiment*.**

DAY 3

1. **Tell students that today they will analyze the results of the bacterial growth experiment they have been running and will use those results to help explain what happened to a high school student who had tuberculosis.**

2. **Organize students into teams and instruct them to collect their bacterial growth plates. While they do this, distribute a copy of Master 3.2, *Discussion Questions for the Bacterial Growth Experiment*, to each student. Tell the teams to draw (or describe) their results on the flow chart on *Bacterial Growth Experiment* first, then refer to those results as they discuss and write answers to the discussion questions.**

   Depending on students' microbiology background, you may need to explain that when a single, microscopic bacterium is placed on an agar plate, it will grow and divide into two progeny cells. Each progeny cell will grow and divide, and so on, until thousands and thousands of individual bacteria are growing right in that spot. At this point, the growth becomes visible to us as a colony of bacteria. Each colony came from a single original bacterium on the plate. When approximately 10,000 or more bacteria are plated, each individual bacterium is close enough to a neighboring bacterium that the colonies they produce merge together, and we observe confluent growth or a "lawn" of bacteria across the plate.

   Move among the teams as they discuss each question and help lead students to the following understandings.

Question 1 Compare the bacterial growth on the two plates from the parental culture (Plates 1 and 2). Which has more growth? Explain why. How do you explain the presence of bacteria on the plate containing kanamycin?

The nutrient agar plate (Plate 1) should show a lawn of bacteria or confluent growth, whereas the plate containing kanamycin should show only 50 to 100 colonies. Students should explain that the antibi otic prevented the growth of most of the bacteria on Plate 2. A simple, straightforward answer is all students need to provide for the last question: The bacteria that grew on Plate 2 were resistant to the antibiotic.

Question 2 Compare the growth on Plates 3 and 4, which you prepared from culture A (without kanamycin). How does the growth on the plates with and without kanamycin appear? What does this tell you about the bacteria grown in culture A?

The plate without kanamycin (Plate 3) should show a lawn of bacter ial growth, whereas the plate with kanamycin (Plate 4) should show 50 to 100 colonies. The results on Plate 3 indicate that a lot of bacteria were growing in the sample plated from culture A. Comparing the results on that plate with the results on Plate 4 indicates that some of the bacteria in the culture (for example, 50 out of 10,000 or more) were resistant to the antibiotic, but most were not.

Question 3 Compare the growth on Plates 5 and 6, which you prepared from culture B (with kanamycin). How does the growth on the plates with and without kanamycin appear? What does this tell you about the bacteria grown in culture B?

Both plates should show a lawn of bacterial growth. This indicates that most or all of the bacteria growing in this culture were resistant to kanamycin.

Question 4 Compare the growth of cultures A and B on Plates 4 and 6 (with kanamycin). Explain how culture B could have so many more resistant bacteria than culture A, even though they both came from the same parental culture.

If, after a minute or two of discussion, students cannot offer an expla nation, suggest that they use their understanding of natural selection to explain the difference in the results on the plates for the two cul tures. They should be able to explain that the environment in culture B (which contained kanamycin) *selected for* the growth of those bacte ria that were resistant to kanamycin. By the time students plated a sample from that culture, all of the bacteria in the sample were resis tant, so they all grew on the plate with kanamycin, resulting in a lawn of bacterial growth (Plate 6). Culture A did not contain kanamycin, so there was no selection for kanamycin resistance, and

most of the bacteria they plated from that culture were not resistant. Thus, most did not grow on the plate with kanamycin (Plate 4).

Question 5 How do you explain the presence of some resistant bacteria in the parental culture and culture A?

To answer this question, students must recognize that bacteria become resistant (for example, through mutation) *before* natural selec tion operates. In other words, the bacteria in the parental strain did not "know" that some of them would be placed in growth medium with kanamycin and "respond" by becoming resistant. Instead, in the parental strain, a few bacteria were already present that were resis tant to kanamycin, even though there was no kanamycin present. Similarly, a few bacteria in culture A were resistant to kanamycin even though no antibiotic was present. When the resistant and nonre sistant bacteria from the parental culture were placed in medium containing kanamycin (culture B), only the resistant bacteria survived and reproduced, passing their kanamycin resistance trait on to their progeny. Soon, virtually all of the bacteria in the culture—the prog eny of the original resistant bacteria—were resistant to kanamycin, as observed on the students' plates.

3. **Convene a brief class discussion in which you clarify any confusion you noted as you circulated among the groups and/or invite students to ask questions about the results of their experiments.**

4. **Tell students that they will watch a young woman named Debi French discuss her battle with tuberculosis. Then they will use the results of their bacterial growth experiments to help explain what happened in her struggle with the disease. Instruct teams to take their copies of the flow chart and *Discussion Questions* with them to the computer stations.**

As they use the results of their bacterial growth experiment to explain what happened to Debi French, students will experience how basic research leads to expla nations for disease and for the success or failure of disease treatment. This understanding leads scientists to pro- pose further research and policies directed at improving public health.

Emphasize that the bacteria in their experiment (*P. fluorescens*) is not the kind that causes tuberculosis (*M. tuberculosis*). *P. fluorescens* does not cause disease in healthy people. Furthermore, the antibiotic kanamycin is not used clinically, so the resistant bacteria cultured in this exercise do not compromise medical treatments. Emphasize, however, that all bacte rial cultures in your class are decontaminated before disposal and that aseptic conditions must be followed in all work with microorganisms.

5. **Distribute a copy of Master 3.3*, Debi's Story: Explaining What Happened,* to each student and tell them to click on *Debi's Story* to start the video. Indicate that students have 20 minutes to answer the questions on *Debi's Story*.**

You may want to emphasize to students that this is a true story, and that Debi herself tells her story on the video.

Organizing student teams at individual computer stations to view Debi French's story will allow them to complete this part of the activity at their own pace. An alternative, if you have the equipment to project the

video from the CD-ROM onto a large screen for whole-class viewing, is to show the first part of the video to the class, then reorganize students into their teams. After the teams have discussed and written answers to the first set of questions on *Debi's Story*, reconvene the class to watch the second part of the video. Instruct students to return to their teams to answer the second set of questions on the handout. Follow this process until students have completed their study of Debi's story.

You may need to remind students of the information they learned about tuberculosis in Activity 1.

6. Convene a whole-class discussion in which you ask several teams to share their responses to the questions on *Debi's Story*. Invite the other teams to add information and disagree with these responses. Then ask students, "What does the Debi French example suggest is an explanation for the re-emergence of diseases like tuberculosis?"

The Debi French example reminds students of the major concept of the activity: One explanation for the re-emergence of infectious diseases is resistance of the causative agent to the treatment that once cured infections of that agent. The important public health issue is avoiding inappropriate use of antibiotics as a way to minimize, or at least delay, the evolution of resistant pathogens.

Students should be able to provide answers such as the following:

Question 1

- **Debi contracted tuberculosis (TB) from** a student in one of her classes who had an active, misdiagnosed case of TB. Debi did not know this student.
- **The symptoms Debi had were** fatigue, weight loss, and a severe, persistent cough.

Question 2

- **The treatment to cure TB is** a combination of several antibiotics. Debi named standard drugs used for TB such as isoniazid and streptomycin.
- **When Debi started the treatment** she initially got better.

Question 3

- **Debi's health began improving when she started the drug therapy for TB because** the bacteria that caused her tuberculosis were killed (or their growth was inhibited) by the drugs she was taking.

Question 4

- **On Valentine's Day 1994, Debi learned** that her tuberculosis was active again.
- **The drugs Debi took to cure her TB were not working because** the bacteria that caused her TB had become resistant to the drugs.

Question 5

- **Debi had a relapse (developed an active case of TB again), even though her health had improved and she was still taking the drugs to cure TB, because** the initial treatment killed some of the disease-causing bacteria, but those that were resistant survived. They contin ued to multiply, passing their resistance on to their progeny. As a

result, the disease in Debi's lungs returned. But now, the disease-causing bacteria were all resistant to the drugs she was taking and the drugs were no longer able to cure her. Point out to students that this is an example of natural selection: The resistant bacteria survived and passed the genes for resistance on to their progeny, whereas the susceptible bacteria did not survive. Soon all or most of the bacterial population, descendants of the resistant organisms, was resistant.

Question 6

- **Debi was finally cured of TB by** taking other drugs that were still able to kill the tuberculosis bacteria and by surgical removal of the upper third of one lung that had the greatest concentration of bacteria.
- **Debi's warning about infectious diseases like TB is** not to be fooled by little bacteria. In her words, they are "stubborn" and develop ways to survive. A scientist would say that bacteria rapidly evolve resistance to the drugs we use to treat infections caused by those organisms.

7. **Point out to students that while it was appropriate to treat Debi with the antibiotics that are usually effective in treating TB, it is not appropriate to use antibiotics to treat illnesses that are caused by viruses. Elicit an explanation of the dangers of this practice by asking a question such as "Although an antibiotic doesn't help you get over a viral infection, if you didn't know any better you might think it wouldn't do any harm. But you know better. Explain what negative consequences can result from inappropriate use of antibiotics."**

   Students should be able to explain that using antibiotics will select for bacteria that are resistant. Subsequent infections—either in the same person or in someone who is infected by the first person—will be caused by disease-causing bacteria that are resistant, and successful treatment will be much more difficult or even impossible. This line of logic requires extrapolation of the ideas students developed from their bacterial growth experiment and the Debi French story, so you may need to help them develop their explanation by giving them additional information and asking probing questions such as "What if the antibi otic taken by a person who has a bacterial infection doesn't kill all of the disease-causing bacteria? What can you say about the bacteria that sur vive?" and "Research experiments have shown that harmless bacteria that become resistant to antibiotics can transfer that resistance to other bacteria, including disease-causing bacteria. How does this help explain why doctors don't want to prescribe antibiotics for viral infections?"

   You may want to tell students that the evolution of antibiotic-resistant pathogens is a problem for treating more diseases than TB. For exam ple, many strains of the organism that causes the sexually transmitted disease gonorrhea (*Neisseria gonorrohoeae*) and most strains of a com mon organism that causes many skin infections (*Staphylococcus aureus*)

are now resistant to penicillin. Students consider a proposal to develop a new treatment for multiple-drug-resistant *Staphylococcus aureus* in Activity 5, *Making Hard Decisions*.

8. **Distribute one copy of Master 3.4, *Antibiotic Concerns*, to each team and assign one of the three statements to each team. Explain that each statement describes an example of an inappropriate or potentially inappropriate use of antibiotics. Instruct the teams to develop a brief public service announcement that would persuade the general public not to use antibiotics inappropriately. The announcement should be something that could be read on the radio, featured in a television commercial, or displayed on a public bulletin board. Collect the announcements and read several to the class; display all of them on a bulletin board in the classroom.**

This step provides an opportunity to evaluate students' understanding of the evolution of antibiotic resistance and its relevance to personal and public health.

Laboratory Preparation for Activity 3

1. *Four weeks before conducting the activity*. Order the following materials from Carolina Biological Supply:

   • *Pseudomonas fluorescens* culture, catalog #AA-15-5255Ä
   • nutrient broth, catalog #AA-78-5360Ä
   • nutrient agar, catalog #AA-78-5300Ä
   • kanamycin, catalog #AA-21-6881Ä

   Allow two weeks for delivery. Carolina Biological Supply will onlyÄ ship live or perishable materials on Mondays, Tuesdays, andÄ Wednesdays.Ä

2. *Two days before conducting the activity*. Prepare the following additional materials:

   • petri dishesÄ
   • capped test tubesÄ
   • sterile 1-ml pipetsÄ
   • pipet pumps or bulbsÄ
   • glass rod spreadersÄ
   • Bunsen burnersÄ
   • alcohol (for sterilizing the glass spreaders)Ä
   • facilities for sterilizing and preparing growth mediaÄ

3. Prepare a stock solution of 25 mg/ml kanamycin in water and filter-steril ize it into a sterile test tube.

4. Prepare nutrient broth medium and nutrient agar plates following the directions on the packages. For medium containing kanamycin, aseptically add 2 ml of the stock kanamycin solution per liter of medium after the medium has cooled (but before the agar solidifies, in the case of plates).

5. Dispense 5-ml aliquots of nutrient broth into sterile, capped test tubes. You will need 2 test tubes of nutrient broth and 1 test tube of nutrient broth con taining kanamycin for each team. You will also need 3 nutrient agar plates and 3 nutrient agar plates containing kanamycin for each team. We recom mend preparing extras to allow for contamination and errors.

6. Inoculate 1 nutrient broth tube with *P. fluorescens* for each team 2 days before Day 1 of the activity (use a 0.1 ml inoculum). Incubate these cultures at 25°C.

If students are unfamiliar with aseptic technique, you will need to provide that instruction before they begin the experiment. You may want to demonstrate these techniques by showing the Day 1 segment of *Bacterial Growth Experiment* on the CD-ROM. This segment shows students completing the first four steps of the experiment and observing aseptic techniques such as using sterile pipets, flaming the open mouth of a test tube before replacing the cap, and sterilizing

and using a glass rod to spread a culture sample on a plate. The video also shows students observing safety practices such as tying back long hair, wearing lab coats and safety goggles, and washing their hands. Hands, equipment, and counter tops should be washed with a commercial, microbiological disinfectant, or with household bleach diluted 30-fold with water. You should also identify a place for students to discard their used cultures and explain that you will decontaminate all materials before disposal.

The *P. fluorescens* that is cultured in nutrient broth or on nutrient agar will grow up in 24 hours; however, the cultures in media containing kanamycin will take two or three days. We recommend that, after 24 hours of incubation, you refrig erate students' cultures in media without kanamycin (broth culture A and plates 1, 3, and 5). This will prevent overgrown cultures that may obscure the results.

All cultures should be decontaminated when students have completed their work. Used cultures should be placed in an autoclave at 1 atmosphere pressure for 15 minutes to kill bacteria. Plastic petri dishes should be placed in heat-resistant plastic bags prior to autoclaving because the dishes will melt and leak. A kitchen pressure cooker can also be used to kill bacterial cultures.